



Supplementary information, Figure S12 Disruption of cofilin 1 by CRISPR/Cas9 induces ciliary phenotypes. One-cell embryos of zebrafish were co-injected Cas9 mRNA (250 pg) and cofilin 1 g RNA (20 pg) or not. (A) Cartoon illustrating the gene structure, the position of the target site and the restriction enzyme (Nco I) cutting site (underlined) in zebrafish *cofilin 1* locus. PAM sites indicated in red. (B) At 48 h after injection, 10 embryos were randomly selected and pooled to extract their genomic DNA for PCR amplification. Mutations were validated with restriction enzyme digestion and Sanger sequencing. Representative sequencing results are shown. Target sequence is underlined; PAM sites are shown in red; mutations are indicated in green. (C, D) Bright-field micrographs show that the zebrafish co-injected with Cas9 mRNA and cofilin 1 g RNA exhibited body curvature and pericardial edema (arrow) at 72 hpf. Scale bar, 500 µm. (E-G) Immunofluorescence with anti-acetylated-tubulin antibody (green) at 8 ss reveals longer cilia in Cas9/cofilin 1-gRNA-treated embryos compared to that of the control (E). Scale bar, 10 µm. Cilia length in KV is measured by Image J software (F). The average number of cilia per KV is also shown (G). Each bar represents mean ± SD of three independent experiments. n, sample size. *** $P < 0.001$; ns, not significant ($P > 0.05$).